Determination of Total Lipid and Lipid Subclasses in Meat and Meat Products

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Current interest in physiological and nutritional activities of the sterol, polyunsaturated fatty acid, and polar lipid fractions of meats and other foods indicates that analytical methods for lipids should be evaluated on their ability to recover and quantitate these classes. Current methods of lipid isolation furnish an extract that is dependent on the solvent(s) used, the type of food material, the temperature of extraction, and the relative proportions of the lipid classes present. Extraction with ethers or other relatively nonpolar solvents removes principally the neutral fats and nonpolar lipids. For an approximation of the crude fat content, such extraction is often sufficient, because the nonpolar fraction generally constitutes over 90% of the total lipids present. The polar lipids include the biochemically important (ω-3) and (ω-6) polyunsaturated fatty acid classes; thus, the method of lipid extraction of food products becomes relevant for a more complete and valuable characterization of their nutritional value. The various methods of lipid determination for meat products are examined for their total recovery of these important lipid groups. A sequential extraction in conjunction with subsequent analytical methods is recommended.

Today, interest in dietary fat is widespread because of either its beneficial or its purportedly harmful effects. This interest suggests a need for more complete labeling of food products for fat content—both *total* fat or lipid content and lipid composition. However, this need is not easily addressed, in part, because official methods used for lipid (fat) analysis only approximate the *total* lipid in meat and meat products, and methods that do report total lipid have not, for various reasons, been adopted as official methods.

To date, no exact definition of the composition of a lipid isolate has been agreed upon. The term lipid has been used traditionally to describe a variety of products that have in common solubility in solvents such as diethyl ether, hexane, chloroform, and/or methanol. A simplified classification of a lipid mixture isolated from muscle tissue covers most classes of compounds accepted as lipids. In this classification, lipids are grouped in 2 broad subclasses: the "simple" or "neutral" lipid class, which includes cholesterol (free and esterified), free fatty acids, and glycerides (mono-, di-, and tri-); and the second, the "complex" or "polar" class, which includes phospholipids and sphingolipids. The simple or neutral fraction also includes minor components, such as wax esters and hydrocarbons. The complex or polar lipid fraction contains most of those compounds (with the exception of cholesterol) that are of increasing interest to health professionals and consumers, e.g., the phospholipids, which contain high levels of the ω-3 and ω-6 fatty acids.

The terms neutral and polar have been used for some time to describe glycerides and phospholipids, respectively; however, *simple* and *complex* have been recommended to describe these subclasses because these terms are thought to have more precise meanings (1). The latter category was derived from the number of hydrolysis products that lipids form. Hydrolysis of a simple lipid such as a triglyceride yields 2 products/mol (glycerol and fatty acids), whereas a complex lipid such as a glycerol phospholipid yields 3 or more types of products (glycerol, fatty acids, phosphate, and an organic base).

Lipid Methodology

When AOAC methods are used for fat analysis, an extract is obtained which is termed "crude" fat (2). This term, although widely used, is not included in the simplified classification described earlier because it is not a clearly defined portion of the fat in meat and meat products, but represents instead only a portion of the *total* lipid recovered by extraction with either ethyl or petroleum ether, the 2 solvents specified for extraction. AOAC method **24.005**, a Soxhlet extraction technique, requires use of ethyl ether, although petroleum ether is sometimes used (2). Fat extractions by this method using the traditional apparatus require about 4–8 h for completion. An alternative method (**24.006–24.008**) uses the Foss-let apparatus, with tetrachlorethane as the extracting solvent (2). This extraction requires only about 30 min. However, although this method is precise compared with the Soxhlet method, it too yields a crude fat extract (3).

An earlier study by Hagan et al. (4) illustrates some of the problems associated with fat extracts obtained with crude lipid methods. Table 1 shows their results for extraction of fat from 3 cuts of beef, which were obtained by varying the extraction and drying methods and the extracting solvents. From these studies, it was established that the results obtained by the AOAC Soxhlet method are dependent on the solvent, the drying method, and the fat content of the sample. The Soxhlet method was also compared in this study with results obtained when the same samples were analyzed by the Bligh and Dyer method (5). The latter method uses a solvent combination of chloroform and methanol and has been used traditionally by analysts who require an intact *total*

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Table 1. Lipid content of beef extracted by several solvent systems and phospholipid content of extracted lipids[a]

Extn method	Solvent	Drying method	Total lipids extracted, %			Ratio, % PL/PL + TG[b]		
			Cut I	Cut II	Cut III	Cut I	Cut II	Cut III
Soxhlet	ethyl ether	freeze dryer	3.98	7.42	1.99	3.0	2.9	6.7
		vacuum oven	4.08	7.01	1.86	4.2	3.6	8.8
		air oven	3.98	7.03	1.82	3.8	3.9	9.6
	petroleum ether	freeze dryer	3.94	7.32	1.83	2.9	3.1	6.7
		vacuum oven	3.97	7.48	1.74	4.5	3.8	7.5
		air oven	4.00	7.56	1.74	3.7	3.2	8.0
	mixed ethers	freeze dryer	3.99	7.28	1.90	3.8	2.8	6.8
		vacuum oven	4.00	7.24	1.74	3.9	4.4	8.6
		air oven	3.94	7.40	1.84	4.1	4.0	7.7
	chloroform	freeze dryer	4.44	8.12	2.35	8.9	7.4	17.8
		vacuum oven	4.37	8.03	2.08	7.2	6.4	15.3
		air oven	4.44	8.25	2.04	7.4	6.9	14.0
Acid hydrolysis-Rohrig	ethyl and petroleum ethers	none	3.81	5.58	1.65	2.8	1.8	6.3
Bligh and Dyer	chloroform–methanol	none	4.60	7.17	2.50	10.1	8.3	18.3

[a] Adapted from ref. 4.
[b] PL = phospholipids; TG = triglycerides.

lipid extract that has not been altered by the extraction process. The chloroform/methanol method, which does not require drying the sample prior to extraction, invariably yields larger recoveries of lipids than does the AOAC Soxhlet method. This difference results in part from a more complete extraction of phospholipids by the Bligh and Dyer method. Table 1 also shows phospholipid recovery for the same samples.

These data indicate that the Soxhlet method gives varying recoveries of phospholipids, depending on the extracting solvent used, but regardless of the solvent, Soxhlet results are quite low compared with those obtained by the Bligh and Dyer method (5).

Methods such as the Soxhlet technique, which isolate a crude fat extract, are used primarily when a value for percent fat content is required for reporting purposes. However, today's consumers, nutritionists, and other health professionals would prefer methods that report total rather than crude fat values. Such methods are particularly needed when the amounts of cholesterol, polyunsaturated fatty acids, and phospholipids in animal-based products are required. The 2 methods traditionally used for determining total lipid were those developed by Bligh and Dyer (5) and Folch et al. (6). Both methods involve homogenization of the tissue, extraction of the resultant mixture in chloroform–methanol solvent systems, filtrations to remove residues, extraction or chromatography to remove nonlipid artifacts, and finally column chromatography to separate lipid classes (1). Although these methods are accurate, they require skilled operators and are time-consuming.

In recent years, our laboratory has developed a new method for lipid extraction, which differs significantly from other techniques and which obviates many of the problems encountered with traditional methodology (7, 8). This "dry column" method is quite versatile in that lipid may be isolated as 3 distinct fractions by simple changes in the solvents used.

A meat sample is placed in a mortar together with an antioxidant, ground with anhydrous sodium sulfate, and then blended with Celite 545. The powdered mixture is transferred to a glass chromatographic column containing a trap material of calcium hydrogen phosphate–Celite 545 (1 + 9). After the column is packed the lipid may be recovered in one of 3 ways: (*1*) Elution of the column with dichloromethane alone results in recovery of the "crude" lipid fraction (7). (*2*) Elution of the column with dichloromethane–methanol (9 + 1) results in recovery of a total lipid extract (7, 8). (*3*) Sequential extraction of the column first with dichloromethane to elute a neutral lipid fraction, and then with dichloromethane–methanol (9 + 1) to elute the polar lipid fraction (8). Lipid thus may be selectively isolated depending on subsequent analytical needs.

In our initial studies, the dry column method was compared with AOAC method 24.005 (2); nearly identical results for crude lipid could be obtained by either method (7). Later comparisons were made with the Folch chloroform–methanol method (8) where muscle tissue of varying fat content (3.4–29.3%) was analyzed by both methods for total lipid (as a sequential extraction) and phospholipid content (Table 2). These results indicate that sequential elution by the dry column method, which gives separate neutral and polar lipid fractions, results in a sum for these 2 fractions equal in amount to that obtained by the Folch method. Moreover, identical results were obtained for the phosphatides in these samples by both methods, and comparisons by thin layer chromatography gave additional verification for the equivalency of results between methods (8). A practical application for sequential separation of lipids in meat analysis is gas chromatography (GC) when a fatty acid profile of the lipids is required. Meat lipids typically are derivatized to methyl esters from a total lipid extract, and are subsequently separated on a GC system. By use of dry column sequential elution, the neutral and polar lipid fractions may be separated individually (9), which allows the analyst to examine the acyl composition of the phospholipid fraction more precisely. This

Table 2. Comparison of lipid extraction methods[a]

Tissue	Dry column (sequential)			Folch
	Neutral fraction, %	Polar fraction (phosphatide), %	Sum total, %	Total (phosphatide), %
Lean pork	4.97	0.82 (0.64)	5.79	5.77 (0.67)
Fatty beef	29.35	0.67 (0.46)	30.02	29.87 (0.50)
Med. beef	10.08	0.72 (0.56)	10.80	10.70 (0.60)
Lean beef	3.43	0.80 (0.69)	4.23	4.38 (0.67)

[a] Adapted from ref. 8 with permission of the American Oil Chemists' Society.

Table 3. Comparison of AOAC Soxhlet (7.056) and dry column methods for extraction of crude and total fat and phosphatide from canned pet foods[a]

	Dry column		AOAC Soxhlet	
	Total fat, %	Phosphatide, %	Crude fat, %	Phosphatide, %
Dog food				
Beef	9.60	0.76	8.65	0.48
Chicken	7.80	0.25	7.03	0.05
Lamb	5.18	0.73	4.50	0.32
Liver	6.41	0.99	5.63	0.48
Cat food				
Beef & liver	10.83	1.37	10.05	0.50
Salmon	9.08	1.43	7.58	0.46
Tuna	2.93	0.89	1.99	0.13

[a] Adapted from ref. 12.

feature is important because the polar lipids contain most of the biologically important polyunsaturated fatty acids. Such an approach was used in this laboratory to separate the lipids from hundreds of muscle samples in a study of dietary factors in beef animals (10).

Zubillaga and Maerker (11) modified the dry column method, which was originally developed for fresh meats, to analyze the lipids in nitrite-treated, heated meat and meat products. They found that subtle changes occurred in the resultant fractions when the sequential dry column procedure developed for fresh meats was used with cooked nitrite-treated meats. Neutral lipids recovered in the dichloromethane fraction were clear, but the polar fraction was bright yellow, a result of pigmented materials in the eluate. These pigmented materials catalyzed lipid oxidations and interfered with evaluation of possible antioxidant activity in the extracts (11). These authors corrected this problem by a slight alteration in the trap material of the chromatographic column, which allowed the polar fraction to be collected free of any pigmented contaminants. Other column modifications by these workers resulted in isolation of pigmented material in the polar fraction from cooked meat as a separate fraction, thus aiding its identification (11). Their efforts have assisted considerably in understanding the effects of oxidation on extracted lipids.

The dry column method has also been used for lipid isolation from foods other than raw and cooked meat and meat products. In a recent study (12), the method was compared with the AOAC crude fat method **7.061–7.062** (2) for canned pet foods. This study was instituted to provide an alternative to the official method where a nonflammable solvent extraction system could be used in place of the ethers needed with the Soxhlet apparatus. Results in Table 3 for various pet foods show that the major difference between the 2 methods is the amount of extractable phosphatide. The higher values for total lipid obtained by the column method result mainly from greater recoveries of phospholipid by this technique. Similar differences would be expected for other canned processed foods.

In addition to processed foods, applicability of the column method to other foodstuffs such as legumes (13) and milk products has been examined. Milk and milk products were extracted by the dry column method, and the results were compared with those obtained by the Roese-Gottlieb (Mojonnier) method for lipid extraction (14). In this study, milks of varying fat content were analyzed initially for total lipid and phospholipid by the 2 methods (Table 4). Slightly higher total lipid values were obtained by the dry column method

Table 4. Comparisons of dry column and Roese-Gottlieb (Mojonnier) methods for lipid extraction from milk[a,b]

	Dry column		Roese-Gottlieb	
	% Total lipid	% Phosphatide	% Total lipid	% Phosphatide
Heavy cream	36.40	0.200	35.81	0.165
Light cream	17.53	0.119	16.87	0.087
Whole raw	4.41	0.039	4.02	0.030
Buttermilk	1.89	0.133	1.43	0.115
Skim	0.83	0.018	0.66	0.015

[a] Adapted from ref. 14 with permission of the American Dairy Science Association.

[b] All samples from same raw milk source.

than by the Roese-Gottlieb method. More important, phosphatides were recovered in higher yields by the former method. This finding is significant because milk contains small amounts of phospholipid and the Roese-Gottlieb method, which uses ammonium hydroxide in the extraction process, may cause alteration or destruction of certain sensitive polar lipids such as phosphatidyl serine (15).

The dry column method was recently applied by Hundrieser et al. (16) to isolate lipids from human milk. This work was undertaken to find an alternative to the Folch or Roese-Gottlieb method. Agreement between the Folch and dry column methods was good. Use of the latter method also avoided the difficulty of time-consuming separations in the Folch method where recalcitrant emulsions are often formed during extraction.

The dry column method also has been modified by other researchers to determine nitrosamines in bacon and other cured meat products (17). The method used by the Food Safety and Inspection Service (FSIS) to determine violative levels of nitrosamines was the lengthy mineral oil distillation–gas chromatographic–thermal energy analyzer screening method (17), which limited analyses to about 6 samples/day/analyst. In comparison studies, the modified dry column method was rapid (20 samples/day/analyst), less susceptible to artifactual nitrosamine formation, and equally as precise as the FSIS method (17). The column procedure was collaboratively studied (18), and adopted by AOAC (19).

Numerous methods are available to the analyst for isolating lipid from meat and meat products. The choice of method, however, will influence the composition of the resultant lipid extract and its suitability for further analytical studies. Although method choice presently may be governed by official reporting requirements, concerns now expressed by consumers and health professionals regarding the completeness of published values for lipid composition of foods may result in efforts to substitute total rather than crude fat methods for such purposes and may lead to a reassessment of the methods currently used for fat analysis.

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